Oral Oncology 60 (2016) 18–24

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

# Prognostic efficacy of combining tumor volume with Epstein-Barr virus DNA in patients treated with intensity-modulated radiotherapy for nasopharyngeal carcinoma

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ARTICLE INFO

*Article history:*
Received 8 April 2016
Received in revised form 17 June 2016
Accepted 18 June 2016
Available online 30 June 2016

*Keywords:*
Intensity-modulated radiotherapy
Tumor volume
Epstein-Barr virus DNA
Prognosis

ABSTRACT

*Objectives:* To evaluate the prognostic effect of combining tumor volume with pre-treatment plasma Epstein-Barr virus DNA (EBV DNA) in patients treated with intensity-modulated radiotherapy (IMRT) for nasopharyngeal carcinoma (NPC).

*Materials and methods:* A total of 180 consecutive NPC patients enrolled in this observational, prospective study and underwent IMRT. Tumor volume was delineated with IMRT planning system and plasma EBV DNA level was quantified by polymerized chain-reaction assay. The effects of tumor volume and EBV DNA level, either alone or in combination, on 5-year overall survival (OS) were cross-compared.

*Results:* The 5-year OS in patients with gross tumor volume of nasopharynx (GTVnx) $\leqslant$ 20 cc and >20 cc was significantly different ($P = 0.001$). The 5-year OS in patients with EBV DNA <6800 copies/mL and $\geqslant$6800 copies/mL was also significantly different ($P < 0.001$). Based on the combination of GTVnx with EBV DNA, the 5-year OS in different subgroups was: low-risk (100%), intermediate-risk (87.8%, 95% CI: 70.6–95.2%) and high-risk (61.3%, 95% CI: 47.9–72.2%). Patients with small tumor volume and high EBV DNA level had a worse prognosis than those with large tumor and low EBV DNA level. Patients with low EBV DNA levels, and either small or large tumor volumes, had favorable prognosis. According to small or large tumor volume, patients with high EBV DNA level were divided into intermediaterisk and high-risk subgroups.

*Conclusion:* Combining tumor volume with pre-treatment plasma EBV DNA level altered survival-risk definition for subgroups of NPC patients and this combination, more than individual factors alone, improved the accuracy of prognostic evaluation.



## Introduction

The current nasopharyngeal carcinoma (NPC) tumor-node-metastasis (TNM) staging system for prognostic prediction was formulated in the era of conventional two-dimensional radiotherapy (2D-RT) [1,2]. With the increasing application of intensity-modulated radiotherapy (IMRT) and induction-concurrent chemotherapy, the survival rate of NPC has constantly improved and the divergence in survival rates of different stages has been reduced gradually [3–6]. A challenge in NPC research is to develop new indicators which, with TNM staging criteria, will increase prognostic precision [7].

Previous studies indicated a significant correlation between tumor volume and survival rate [8]. Since tumor volume can be accurately delineated under the current IMRT planning system, it is one of the greatest concerns as a prognostic factor [9]. Some studies even found that tumor volume was superior to TNM staging for predicting survival [9–11].

Given the biological heterogeneity of NPC, the present staging system, even in combination with tumor volume, remains inadequate for predicting prognosis [12–14]. It has been well established that Epstein-Barr virus DNA (EBV DNA) level is closely associated

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http://dx.doi.org/10.1016/j.oraloncology.2016.06.013
1368-8375/

with the prediction of distant metastasis and overall survival (OS) of patients [15–20].

Most previous studies considered tumor volume or plasma EBV DNA (EBV DNA) separately in prognostic analysis. However, there is little data regarding the prognostic value of tumor volume combined with plasma EBV DNA. To date, there have been three series of studies on the relationship between pre-treatment EBV DNA and tumor volume [21–23]. The first study of 57 patients with advanced NPC who were treated with 2D-RT, found a positive correlation between pre-treatment plasma EBV DNA levels and tumor volumes delineated in diagnostic MRI [21,24]. A more recent study of 165 cases with NPC treated with IMRT confirmed this conclusion [22]. However, prognostic analyses in this study included only EBV-DNA and showed a significant difference between groups of patients with negative and positive EBV-DNA.

The third study included 69 NPC patients treated with heterogeneous radiotherapy protocols (49 with 2D-RT, 20 with IMRT) [23]. The pre-treatment plasma EBV DNA level was significantly associated with tumor volume. Two threshold values of EBV DNA level (20,000 copies/mL of pre-treatment for metastasis and 0 copies/mL of the end of RT) were used to compare patient survival. However, there were limitations in this study: radiotherapy protocols were heterogeneous and sample size was limited.

All three studies provided valuable insights into the correlation of tumor volume with EBV DNA. We deduced that combining tumor volume with EBV DNA may allow us to identify different risk subgroups for personalized treatment strategy. This observational, prospective study aims to compare prognostic effect using tumor volumes and pre-treatment plasma EBV DNA levels, either alone or in combination, for NPC patients treated with IMRT.

## Materials and methods

### *Patients*

Between February 2006 and December 2009, 186 new NPC patients at Sun Yat-Sen University Cancer Center were admitted in accordance with the inclusion criteria: (1) 2002 UICC stage I–IVb; (2) Karnofsky score $\geqslant$80 points; (3) age $\leqslant$75 years old; (4) blood routine, liver and kidney function tests were normal before treatment; (5) receiving IMRT. Patients with previous radiotherapy or chemotherapy, a history of previous or synchronous malignancy, active peptic ulcer, hypertension, diabetes, or who did not meet follow-up requirements more than twice (6 patients) were excluded. The remaining 180 cases qualified for this study. Approval of the Institutional Ethics Board was obtained.

The pre-treatment assessment included routine physical examination, in particular of the head, neck, and nasopharynx. All patients received MRI plain and enhanced scan of the head and neck. Sixty-seven patients received PET/CT scan. The assessment also included chest radiography (or chest CT for patients suspicious of lung metastasis), abdominal ultrasound, systemic ECT bone scan for N3 stage patients, blood routine, liver and kidney function tests, EB virus serological antibody assay (IgA-VCA, IgA-EA), and EBV-DNA quantification. Based on MRI, all patients were staged according to the 2002 AJCC/UICC staging.

### *Treatment*

All patients were given IMRT using Mimic (NOMOS) [5,6]. The prescribed dose for primary lesion was 68–70 Gy in 30–32 fractions. The dose for cervical lymph node metastases was 60–68 Gy in 30 fractions and the dose for the cervical lymphatic area was 54 Gy in 30 fractions. Patients at stages III, IVa, and IVb received chemotherapy and radiation therapy (CRT) according to institutional protocols [5]. The CRT regimens included concurrent chemotherapy (cisplantin weekly or on days 1 and 29) or 2–3 cycles induction chemotherapy (cisplantin and 5-fluorouracil) followed by concurrent chemotherapy.

### *Tumor volume definition*

All patients received CT simulation scan (Siemens Plus4) at the radiotherapy position. The images included plain and enhanced CT scan. The scope of each scan was from the top of the head to 2 cm below sub-clavicle head, with 3 mm slice thickness. Contouring of targets and organs at risk was performed under IMRT planning system (NOMOS) according to the institutional treatment protocol [5]. The contouring was in the fusion images of CT with MRI or PET/CT. Treatment plans were reviewed and approved by three radiation oncologists. The gross tumor volumes (GTV) were calculated automatically under the IMRT planning system. We reported tumor volumes as GTV for nasopharynx (GTVnx) and GTV for lymph nodes (GTVnd) [5]. Retropharyngeal lymph nodes were incorporated in GTVnx.

### *Quantification of EBV DNA*

Plasma EBV DNA levels were measured before treatment. Plasma EBV DNA was extracted by QIAmp DNA Blood Mini kit (QIAgen) and approximately 200–400 μl/column (setting of QIAamp Kit) withdrawn from each sample. Circulating EBV-DNA level was measured using a real-time q-PCR system that amplified a DNA segment in the Bam HI-W fragment region of the EBV genome. Amplification data was collected using ABI Prism 7900 Sequence Detector (Applied Biosystems). The undetectable EBV signals in the sample were set at 0 copy/mL.

### *Follow-up*

Therapeutic effect was assessed at the completion of treatment. Within the first 2 years after radiotherapy, follow-up assessments were repeated every 3–6 months. After 2 years, the follow-up interval was increased to every 6–12 months. The details of follow-up assessments were the same as in pre-treatment.

### *Statistical method*

Receiver operating characteristic (ROC) curve analysis was performed to select the optimal threshold for GTVnx, GTVnd, and EBV DNA. Data processing was performed using SPSS16.0 statistical software. The 5-year survival and local control rates were calculated and constructed using the Kaplan-Meier method. The log-rank test was conducted to compare the 5-year OS curves in various groups and subgroups and bilateral statistical analyses were used ($P < 0.05$ was considered statistically significant).

## Results

### *Patient demographics*

The clinical characteristics of the 180 NPC patients are listed in Table 1. Forty-four patients with Stages I and II disease received radiotherapy alone. Of 136 patients at stages III, IVa, and IVb, 99 patients received DDP+5-FU induction and concurrent DDP chemotherapy and 37 patients received concurrent DDP chemotherapy alone. Due to intolerance toxicity, chemotherapy was terminated for 5 patients.

The median follow-up time was 67 months (range: 13–116 months). One hundred and seventy-one patients achieved